

UC Irvine

UC Irvine Previously Published Works

Title

Direct commissural connections to the basket cells of the hippocampal dentate gyrus: anatomical evidence for feed-forward inhibition.

Permalink

<https://escholarship.org/uc/item/2nv0095n>

Journal

Journal of neurocytology, 13(2)

ISSN

0300-4864

Authors

Seress, L
Ribak, CE

Publication Date

1984-04-01

DOI

10.1007/bf01148116

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Direct commissural connections to the basket cells of the hippocampal dentate gyrus: anatomical evidence for feed-forward inhibition

LÁSZLÓ SERESS* and CHARLES E. RIBAK

Department of Anatomy, University of California, Irvine 92717, USA

Received 21 October 1983; accepted 4 November 1983

Summary

After lesions were placed in the hippocampal commissures, degenerating terminals could be localized above, inside and beneath the granule cell layer of the contralateral dentate gyrus. The terminals formed asymmetric synapses with spines, dendritic shafts and somata of granule cells. Degenerating terminals also formed synapses with dendrites and somata of basket cells identified by the Golgi–electron microscope technique. These basket cells were located either at the hilar border of the granule cell layer or in the molecular layer and each formed an axonal plexus around the somata and proximal dendrites of granule cells. These observations provide an anatomical basis for the recently described feed-forward inhibition in this brain region.

Introduction

The commissural pathway to the dentate gyrus arises from neurons in the contralateral hilus (Laurberg, 1979; West *et al.*, 1979; Berger *et al.*, 1981; Voneida *et al.*, 1981) and terminates both above and below the granule cell layer (Blackstad, 1965; Laatsch & Cowan, 1966; Gottlieb & Cowan, 1973; Hjorth-Simonsen & Laurberg, 1977; Kishi *et al.*, 1980). Specifically, the terminals of this commissural pathway are found in the lowest one-third of the molecular layer and scattered diffusely throughout the hilus. These terminals form asymmetric synapses with spines and dendrites that are probably derived from granule cells and hilar neurons.

Previous anatomical studies have not described any synaptic relationships between the commissural axons and the basket cells of the dentate gyrus. Five types of basket cells occur in the dentate gyrus and they have somata located either in the granule cell layer or within 30 to 50 μm of this layer (Seress, 1978; Seress & Pokorny, 1981; Ribak & Seress, 1983). Using a combined Golgi–electron microscopic method that utilizes gold

*Present address: Department of Physiology, University Medical School, Pécs, Hungary.

toning of silver impregnated neurons, we recently demonstrated that the axons of basket cells form axosomatic and axodendritic symmetric synapses with granule cells (Ribak & Seress, 1983). The localization of glutamate decarboxylase with immunocytochemical methods to basket cells (Ribak *et al.*, 1978; Seress & Ribak, 1983) indicates that these neurons mediate GABAergic inhibition of granule cells. These findings are pertinent because recent electrophysiological observations show a feed-forward inhibition of granule cells following stimulation of the commissural pathway (Buzsáki & Eidelberg, 1981). A neuronal circuit that could explain such inhibition would involve excitatory commissural axons that synapse with inhibitory basket cells which inhibit granule cells. A combined Golgi-electron microscopic and degeneration method was utilized in the present study to determine whether commissural axons synapse with identified basket cells, and thereby provide anatomical data in support of a feed-forward inhibitory circuit in the hippocampal dentate gyrus. A preliminary report of these results has been presented (Ribak & Seress, 1982).

Materials and methods

Twenty-five young albino rats (30–35 days, 180–200 g) were used for these studies because neurons of such rats are better impregnated than those of older rats. Rats were anaesthetized with chloral hydrate and placed in a stereotaxic head holder. The hippocampal commissures were surgically cut on the right side with a stainless steel knife. Two or four days after the surgery, the rats were anaesthetized and their brains were fixed by transcardiac perfusion with a solution containing 4.0% paraformaldehyde, 1.0% glutaraldehyde and 0.002% calcium chloride in a 0.12 M phosphate buffer at pH 7.2. The perfused animals were stored overnight in the refrigerator. On the following day, the brains were removed and the left hippocampus was dissected away from the rest of the brain. The extent of the commissural cut was determined in 100 μ m thick Vibratome sections. Specimens were obtained from only those animals which displayed an intact fornix in the left hemisphere, the side contralateral to the lesion.

The left hippocampus was processed according to the Golgi-electron microscopic method of Fairén *et al.* (1977). Briefly, the entire hippocampus was rinsed in an osmium dichromate solution (1 g of osmium tetroxide and 12 g of potassium dichromate in 500 ml of distilled water) and kept in 50 ml of this solution in the dark for four days. Then, the tissue was washed briefly with 0.75% silver nitrate and stored in this solution for three days. After the impregnation, the tissue was processed through 20, 40, 60, 80 and 100% solutions of glycerol before being cut.

Blocks of impregnated tissue were embedded in agar and sectioned with the Sorvall tissue chopper at 100 μ m. Sections were collected on slides, coverslipped and examined in the light microscope. Basket cells in the dentate gyrus were identified, drawn with a Zeiss microscope equipped with a drawing tube and photographed.

Pieces of sections that contained impregnated basket cells were hydrated to distilled water through a series of glycerol solutions. Then, they were placed into a chilled 0.05% oxalic acid for 2 min. Then the sections were brought to room temperature and placed into a 1% solution of thiosulphate for 1 h. Afterwards the sections were processed for electron microscopy using a routine schedule that included post-staining with 2% osmium tetroxide, rapid dehydration and embedding in Epon. Serial thin sections were taken of the somata and proximal dendrites of

basket cells that were located in the region of commissural axon terminations. All sections were stained with uranyl acetate and lead citrate before examination in the electron microscope.

Results

Degenerating commissural axon terminals

Following either the two- or four-day survival periods, degenerating axon terminals were observed in the hippocampal dentate gyrus in the hemispheres contralateral to the cut hippocampal commissures. These terminals displayed a marked increase in the electron density of their axoplasm, a loss of synaptic vesicles and swollen mitochondria (Figs. 1, 2). Astrocytic processes usually surrounded the degenerating axon terminal and contacted portions of the postsynaptic structure (Fig. 1). At any individual survival time, terminals were found that displayed early and late signs of degeneration. Thus, the commissural terminals did not appear to degenerate in a synchronous manner.

The number of degenerating terminals was greater from preparations with the four-day survival period than from those with the two-day period. However, most of the degenerating terminals at this longer survival time were displaced away from postsynaptic structures and were completely surrounded by glial processes. For this reason, the two-day survival time was chosen as most appropriate as it yielded numerous degenerating commissural axons that had recognizable synaptic junctions.

Degenerating axon terminals were observed in all three layers of the dentate gyrus. Most of these terminals were found in the inner third of the molecular layer where they formed axospinous and axodendritic synapses (Fig. 1). Some of these terminals formed synapses with identified granule cell dendrites (Fig. 3). Other degenerating terminals occurred in the granule cell layer and in that part of the hilus 50–70 μm beneath the granule cell layer. A few degenerating terminals were also observed in the deeper regions of the hilus (Fig. 2).

Identification of basket cells that are postsynaptic to commissural terminals

The Golgi–electron microscopic method facilitated the synaptic analysis of individual basket cells and their processes by allowing for the identification of these cells in light microscopic preparations prior to their examination with the electron microscope (Figs. 4, 5). Seven impregnated basket cells were analysed in the present study and degenerating commissural axons were observed to form synapses with their somata and dendrites. In addition, several non-impregnated somata of basket cells were encountered and they were also contacted by degenerating commissural terminals. The examples that are illustrated were drawn from two of the five types of basket cell: pyramidal and fusiform types (Ribak & Seress, 1983).

The somata and dendrites of the analysed basket cells displayed the same ultrastructural characteristics that have been previously described (Ribak & Anderson, 1980; Ribak & Seress, 1983). These features included a large nucleus that displayed an

intranuclear rod or sheet and extensive nuclear infoldings and a large shell of perikaryal cytoplasm that contained an abundance of cisternae of granular endoplasmic reticulum and Golgi complex as well as numerous mitochondria, ribosomes and lysosomes (Fig. 5). The dendrites of basket cells lacked spines (Figs. 6, 8) and were contacted by axon terminals that made both asymmetric and symmetric synapses. These features aided in the identification of gold-labelled basket cells in thin sections.

Degenerating commissural axon terminals formed synapses with both somata and dendrites of basket cells. The basket cell type contacted most frequently on its soma was the molecular layer type. This finding was not surprising since most of the degenerating terminals of commissural axons are found in the molecular layer, the site of the somata of this basket cell type. The other types of basket cells have somata located within or beneath the granule cell layer where fewer degenerating commissural terminals are observed. Indeed, only one of these other basket cells received an axosomatic contact. The dendrites of basket cells are also contacted by degenerating terminals of commissural axons. Both basal dendrites in the hilus (Figs. 7, 8) and apical dendrites in the molecular layer are contacted by degenerating terminals.

Fig. 1. Electron micrograph of a degenerating axon terminal that forms an asymmetric synapse (arrows) with a spine located about 70 μm above the granule cell layer. $\times 39\,000$.

Fig. 2. Electron micrograph of a degenerating terminal that forms asymmetric synapses (arrows) with two dendrites in the hilus. Note the increased density of the axoplasm. $\times 35\,000$.

Fig. 3. Electron micrograph of a gold-labelled granule cell dendrite (D) with a spine that is in close contact with a degenerating axon terminal (arrow). A normal terminal (t) forms an asymmetric synapse with this same spine. $\times 50\,000$.

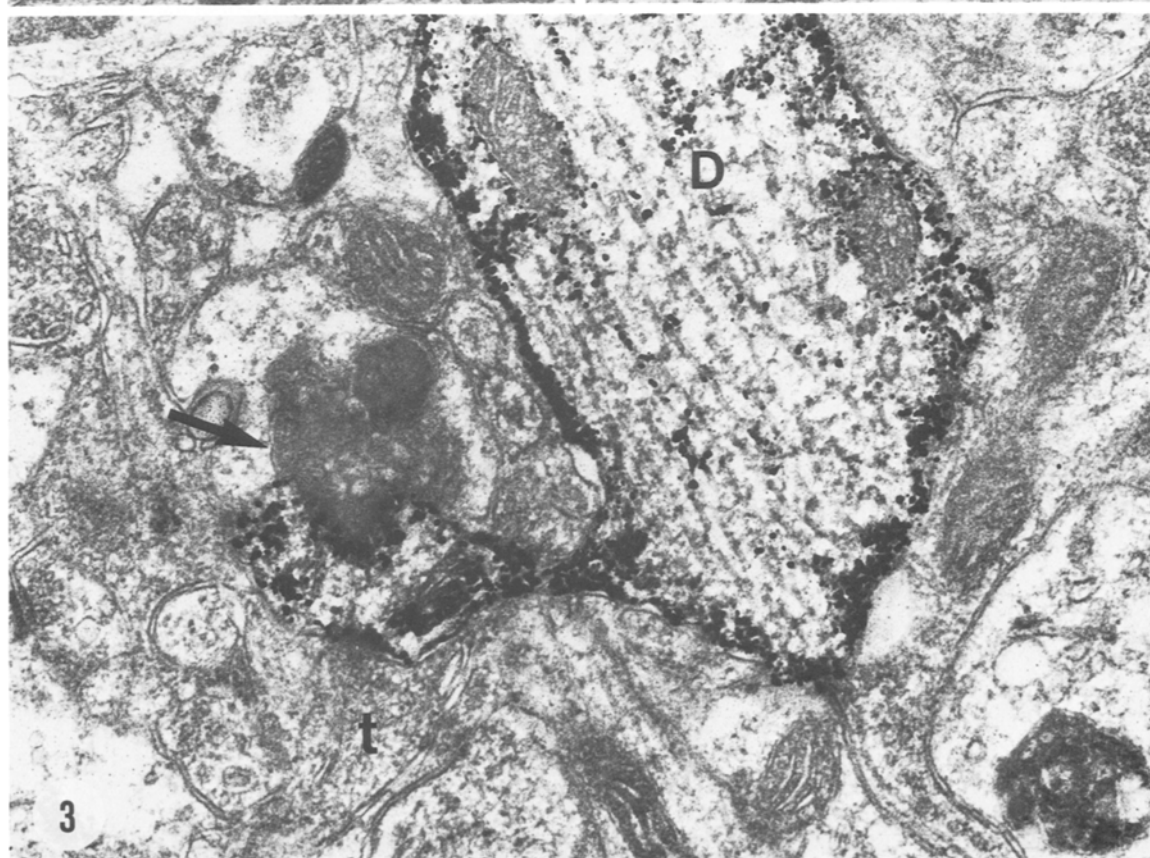
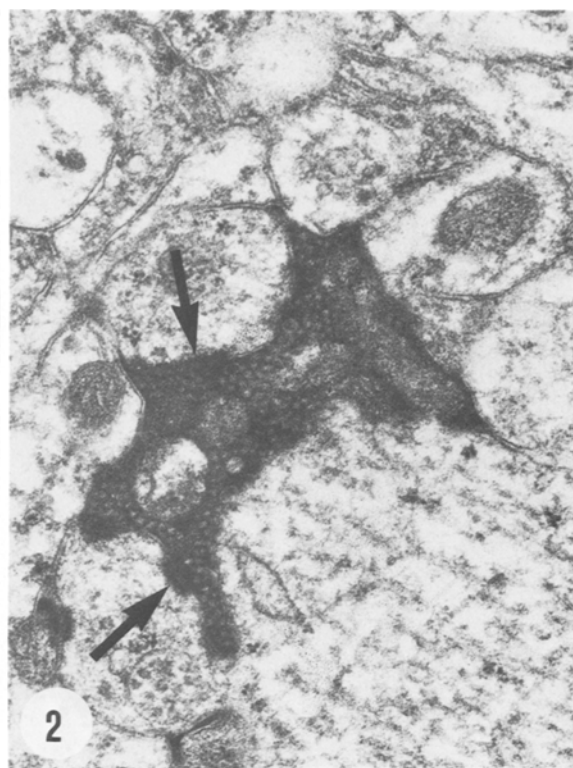
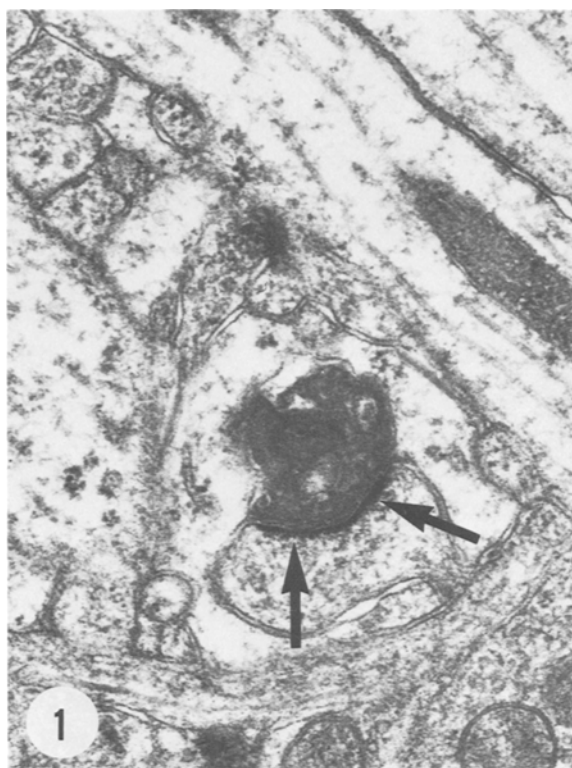
Fig. 4. Light micrograph of a fusiform basket cell that is embedded in Epon. The oval-shaped soma of this cell is located within the granule cell layer (GL). Basal dendrites (arrows) extend into the hilus (H) while a single prominent apical dendrite enters the molecular layer (ML). $\times 900$.

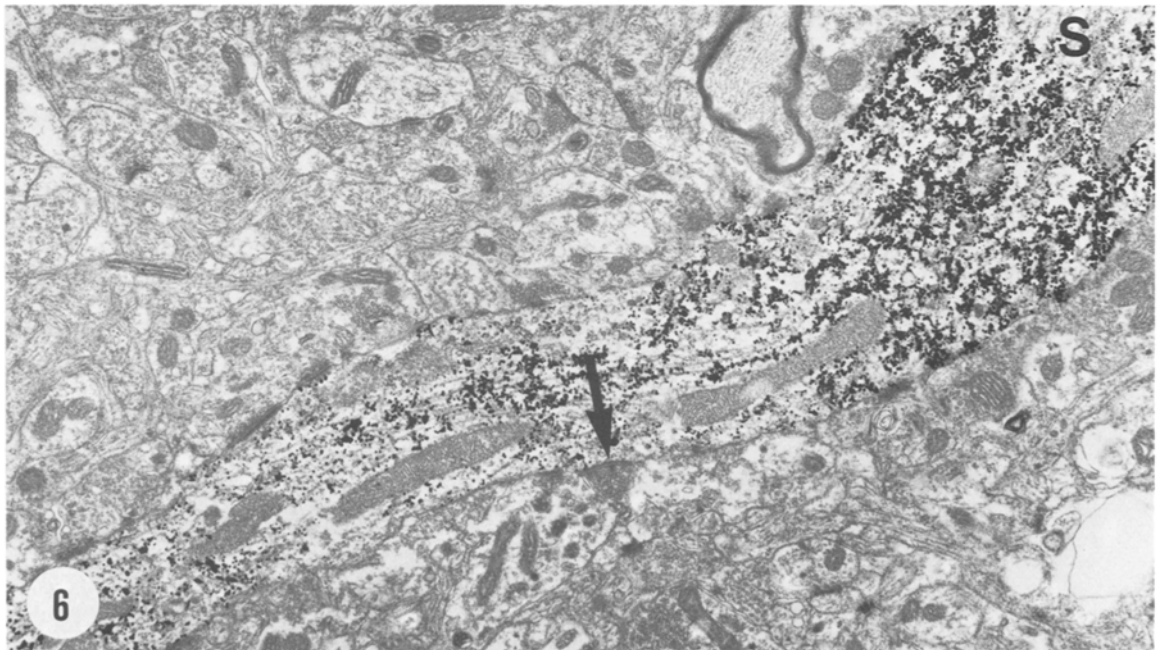
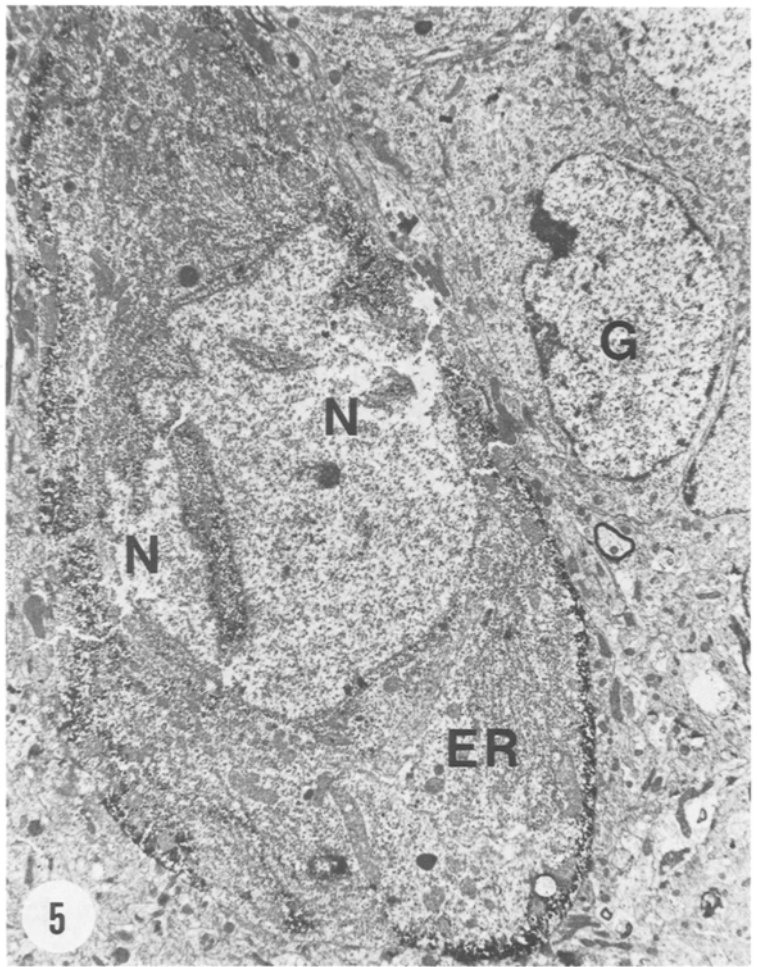
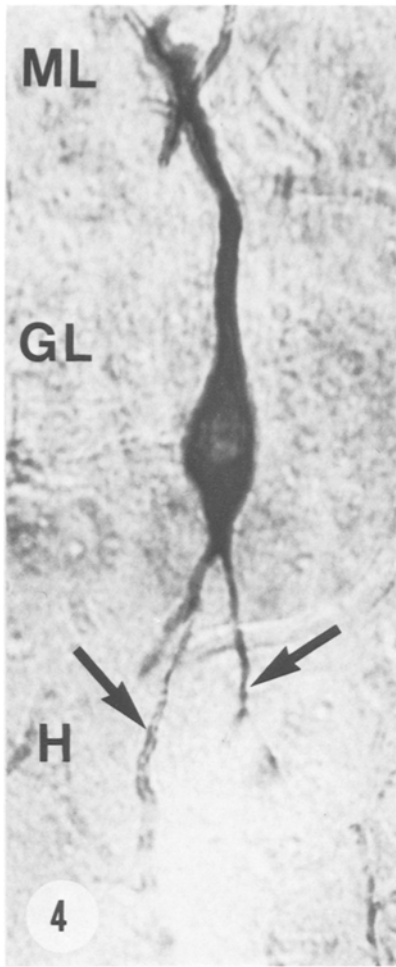
Fig. 5. Electron micrograph of the soma of the gold-labelled fusiform basket cell shown in Fig. 4. The nucleus (N) of this cell displays deep infoldings. The gold label appears concentrated beneath the plasma membrane and allows for the identification of a large Nissl body (ER) in the thick shell of perikaryal cytoplasm. The large size of this basket cell soma can be contrasted with an adjacent granule cell soma (G). $\times 7000$.

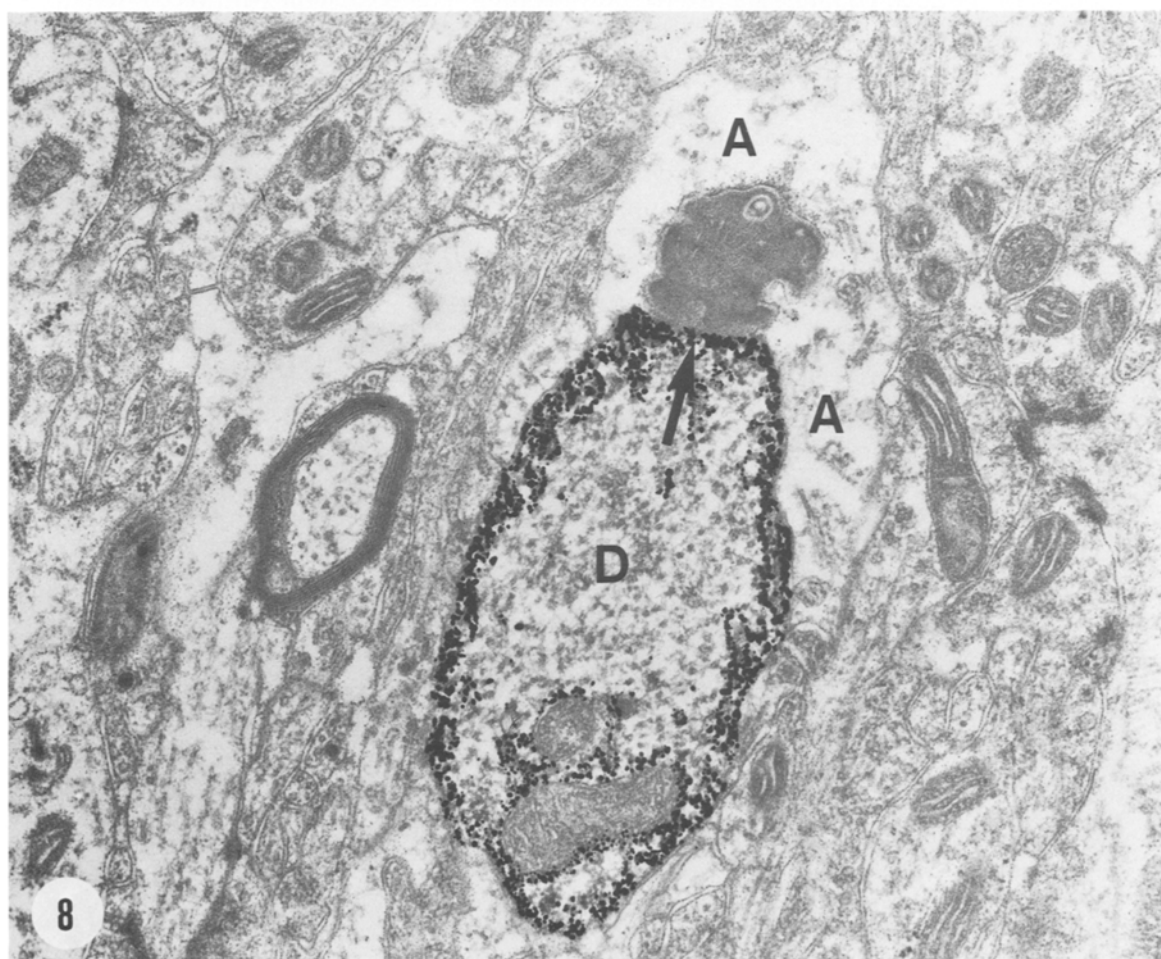
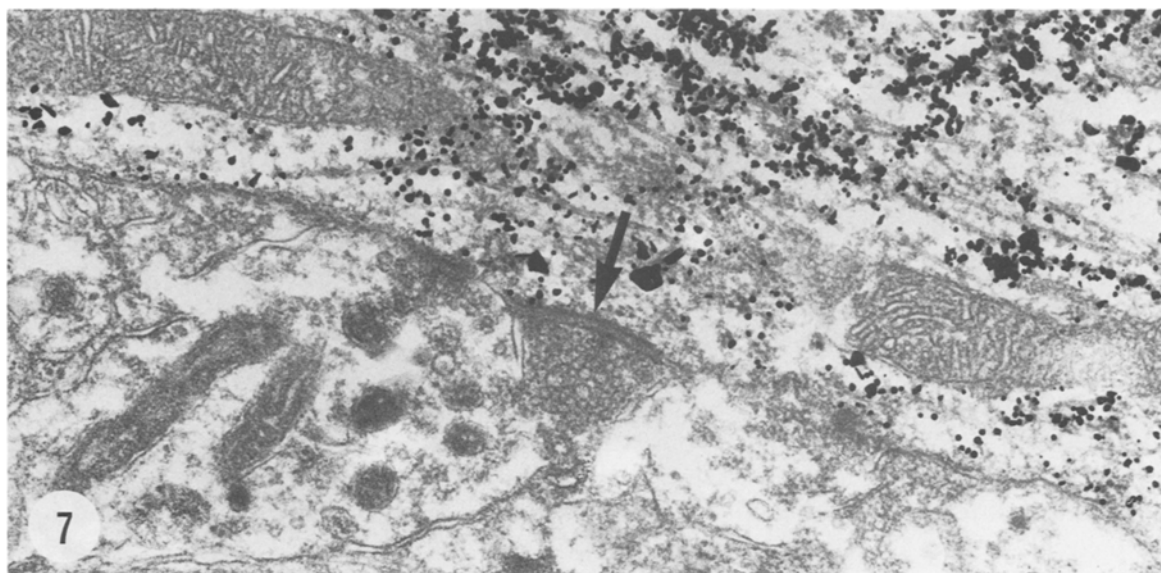
Fig. 6. Electron micrograph of one of the basal dendrites of this fusiform basket cell as it arises from the soma (S). A degenerating terminal forms a synapse (arrow) with this smooth dendrite. $\times 14\,000$.

Fig. 7. Enlargement of the degenerating terminal that forms a synaptic contact (arrow) with the basal dendrite of the fusiform basket cell shown in Fig. 6. $\times 37\,000$.

Fig. 8. Electron micrograph of an impregnated basal dendrite (D) of a pyramidal basket cell. A degenerating terminal is directly apposed to the dendrite, where gold particles (arrow) obscure the synaptic cleft and postsynaptic density. A process (A) from an astrocyte surrounds most of the degenerating terminal and a portion of the labelled dendrite. $\times 32\,000$.







Discussion

In the present study, axons of neurons in the contralateral hilus were lesioned as they passed through the hippocampal commissures causing their terminals to degenerate in the dentate gyrus. The appearance of these degenerating terminals and their synaptic relationships with spines and dendrites in the molecular layer and hilus are consistent with previous reports (Blackstad, 1965; Laatsch & Cowan, 1966; Hjorth-Simonsen & Laurberg, 1977). Since most, if not all, of the spines found in the molecular layer arise from granule cell dendrites, the commissural axons have been assumed to contact granule cells. Our results using a combined Golgi-electron microscopic method have confirmed this assumption in that gold-labelled granule cells display synapses between their spines and degenerating commissural axons.

Commissural axons terminate in the granule cell layer as well as in the hilus and molecular layer. The terminals in the granule cell layer represent a relatively small number of the total commissural axons and this finding is consistent with recent data from this laboratory using anterograde transport of horseradish peroxidase to label commissural terminals (Seroogy *et al.*, 1983). Briefly, these latter results showed that most commissural axons contact the proximal dendrites of granule cells, but some form synapses directly onto their somata. Such axosomatic synapses derived from commissural axons would have a larger influence on the firing rate of granule cells than those axons terminating on the dendrites. The significance of these few synapses is unclear since commissural axons in the granule cell layer appear to be branches from the main axons that course either above or below this layer (Seroogy *et al.*, 1983).

The most significant finding of the present study is that commissural axons form synapses directly with basket cells of the hippocampal dentate gyrus. Our previous results showed that basket cell dendrites in the inner molecular layer are contacted by terminals that also form synapses with neighbouring dendritic spines (Ribak & Seress, 1983). Thus, the smooth apical dendrites of basket cells that ramify in the molecular layer with the spinous dendrites of granule cells are in a position to receive the same afferent systems that synapse with the granule cells. The present study confirms this notion. In addition, commissural axons also form synapses with the somata and basal dendrites of basket cells that are located in the granule cell layer and hilus, respectively. Therefore, the commissural axons contact basket cells in every layer of the dentate gyrus.

The function of this connection between commissural axons and basket cells can be assessed with other knowledge of the circuitry of basket cells (see Fig. 9). Our previous study of basket cells in Golgi-electron microscopic preparations demonstrated two important neuronal connections (Ribak & Seress, 1983). First, the axons of basket cells form symmetric synapses with the somata and dendrites of granule cells. Second, the basket cell dendrites in the hilus are contacted by the mossy fibre axon collaterals of granule cells. These data indicate a reciprocal synaptic relationship between basket cells and granule cell populations. However, we were unable to determine if the same basket

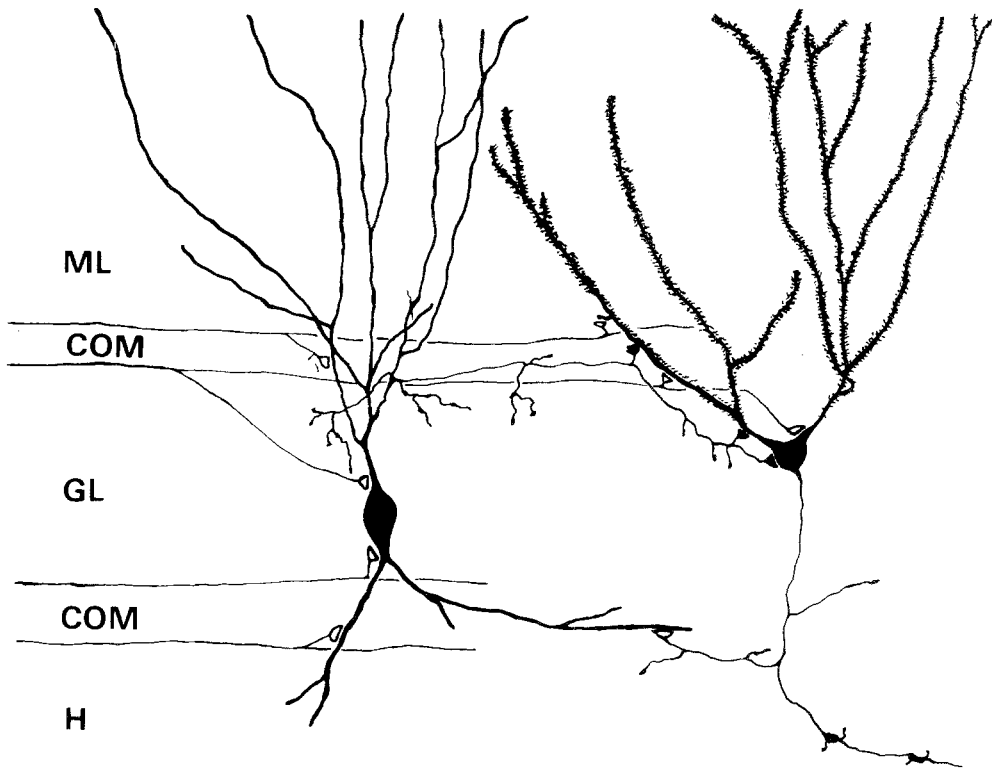


Fig. 9. Summary diagram of findings from this and an earlier (Ribak & Seress, 1983) study that indicate commissural axons (COM) synapse with basket cells (left side) and granule cells (right side). These commissural axons distribute mainly in the molecular layer (ML) where they contact the smooth dendrites of basket cells and the spinous dendrites of granule cells. Other commissural axons terminate in the granule cell layer (GL) where somata are contacted while others synapse in the hilus (H) on basal dendrites of basket cells. In addition to these connections, the diagram illustrates the axon terminals (filled terminals) of the basket cell. These terminals contact the proximal dendrites and somata of granule cells. These two connections may underlie feed-forward inhibition in the dentate gyrus. Finally, the collaterals of granule cell axons make synaptic contact with the basal dendrites of basket cells, a finding recently described as an anatomical basis for feedback inhibition. This diagram represents the populations of neurons in the granule cell layer and does not suggest that one basket cell is reciprocally connected to one granule cell.

cell which forms synapses with an individual granule cell receives synaptic input from that same granule cell. Nonetheless, these findings are consistent with previous immunocytochemical (Barber & Saito, 1976; Ribak *et al.*, 1978; Seress & Ribak, 1983) and physiological (Andersen, 1975) data which indicate that feedback inhibitory mechanisms are mediated via mossy fibre collaterals which synapse with GABAergic basket cells. The findings of the present study provide an anatomical basis for feed-forward inhibition in the dentate gyrus. Such a mechanism has been demonstrated in

physiological studies where excitation of commissural axons causes basket cell activation that inhibits granule cells (Buzsáki & Eidelberg, 1981; Douglas *et al.*, 1983). A similar circuit was recently described with anatomical methods in the CA 1 region of the guinea pig hippocampus (Frotscher & Zimmer, 1983). Therefore, feed-forward inhibition appears as a regular feature of the hippocampus.

Acknowledgements

The authors gratefully acknowledge Y. Jhurani, Y. Kim and M. Brundage for technical assistance and N. Sepion for typing the manuscript. This work was supported by grant BNS 80-23606 from the National Science Foundation.

References

- ANDERSEN, P. (1975) Organization of the hippocampal neurons and their interconnections. In *The Hippocampus*, Vol. 1, *Structure and Development* (edited by ISAACSON, R. L. & PRIBRAM, K. H.), pp. 155–75. New York: Plenum Press.
- BARBER, R. & SAITO, K. (1976) Light microscopic visualization of GAD and GABA-T in immunocytochemical preparations of rodent C.N.S. In *GABA in Nervous System Function* (edited by ROBERTS, E., CHASE, T. N. & TOWER, D. B.), pp. 113–32. New York: Raven Press.
- BERGER, W. B., SEMPLE-ROWLAND, S. & BASSETT, J. L. (1981) Hippocampal polymorph neurons are the cells of origin for ipsilateral association and commissural afferents to the dentate gyrus. *Brain Research* **215**, 329–36.
- BLACKSTAD, T. W. (1965) Mapping of experimental axon degeneration by electron microscopy of Golgi preparations. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **67**, 819–934.
- BUZSÁKI, G. & EIDELBERG, E. (1981) Commissural projection to the dentate gyrus of the rat: evidence for feed-forward inhibition. *Brain Research* **230**, 346–50.
- DOUGLAS, R. M., MCNAUGHTON, B. L. & GODDARD, G. V. (1983) Commissural inhibition and facilitation of granule cell discharge in fascia dentata. *Journal of Comparative Neurology* **219**, 285–94.
- FAIRÉN, A., PETERS, A. & SALDANHA, J. (1977) A new procedure for examining Golgi impregnated neurons by light and electron microscopy. *Journal of Neurocytology* **6**, 311–37.
- FROTSCHER, M. & ZIMMER, J. (1983) Commissural fibers terminate on non-pyramidal neurons in the guinea pig hippocampus – a combined Golgi–EM degeneration study. *Brain Research* **265**, 289–93.
- GOTTLIEB, D. I. & COWAN, W. M. (1973) Autoradiographic studies of the commissural and ipsilateral association connections of the hippocampus and dentate gyrus of the rat. I. The commissural connections. *Journal of Comparative Neurology* **149**, 393–422.
- HJORTH-SIMONSEN, A. & LAURBERG, S. (1977) Commissural connections of the dentate area in the rat. *Journal of Comparative Neurology* **174**, 591–606.
- KISHI, K., STANFIELD, B. B. & COWAN, W. M. (1980) A quantitative EM autoradiographic study of the commissural and associational connections of the dentate gyrus in the rat. *Anatomy and Embryology* **160**, 173–86.
- LAATSCH, R. H. & COWAN, W. M. (1966) Electron microscopic studies of the dentate gyrus of the rat. II. Degeneration of commissural afferents. *Journal of Comparative Neurology* **130**, 241–62.
- LAURBERG, S. (1979) Commissural and intrinsic connections of the rat hippocampus. *Journal of Comparative Neurology* **184**, 685–708.

- RIBAK, C. E. & ANDERSON, L. (1980) Ultrastructure of the pyramidal basket cells in the dentate gyrus of the rat. *Journal of Comparative Neurology* **192**, 903–16.
- RIBAK, C. E. & SERESS, L. (1982) Commissural axons synapse with identified basket cells in the rat dentate gyrus: A combined degeneration–Golgi–electron microscopic study. *Society for Neuroscience* **8**, 836 Abs.
- RIBAK, C. E. & SERESS, L. (1983) Five types of basket cells in the hippocampal dentate gyrus. A combined Golgi and electron microscopic study. *Journal of Neurocytology* **12**, 577–97.
- RIBAK, C. E., VAUGHN, J. E. & SAITO, K. (1978) Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Research* **140**, 315–32.
- SERESS, L. (1978) Pyramid-like basket cells in the granular layer of the dentate gyrus in the rat. *Journal of Anatomy* **127**, 163–68.
- SERESS, L. & POKORNY, J. (1981) Structure of the granular layer of the rat dentate gyrus. A light microscopic and Golgi study. *Journal of Anatomy* **133**, 181–95.
- SERESS, L. & RIBAK, C. E. (1983) GABAergic cells in the dentate gyrus appear to be local circuit and projection neurons. *Experimental Brain Research* **50**, 173–82.
- SEROOGY, K. B., SERESS, L. & RIBAK, C. E. (1983) Ultrastructure of commissural neurons of the hilar region in the hippocampal dentate gyrus. *Experimental Neurology* **82**, 594–608.
- VONEIDA, T. J., VARDARIS, R. M., FISH, S. E. & REIHELD, C. T. (1981) The origin of the hippocampal commissure in the rat. *Anatomical Record* **201**, 91–103.
- WEST, J. R., NORNES, H. O., BARNES, C. L. & BRONFENBRENNER, M. (1979) The cells of origin of the commissural afferents to the area dentata in the mouse. *Brain Research* **160**, 203–15.